

CLAIMS

1. A method of identifying a polynucleotide fragment of a gene that encodes an antigen recognized by an immune effector cell, comprising:

5 (a) providing a first cell that expresses an antigen recognized by the immune effector cell and having an identified major histocompatibility complex (MHC) restriction and one or more second cells having a compatible major histocompatibility complex (MHC) to the first cell but which does not express antigen;

10 (b) identifying polynucleotides encoding a peptide sequence motif in the antigen displayed by antigen presenting cells and recognized by the immune effector cell;

(c) identifying polynucleotides which are aberrantly expressed by the first cells as compared to one or more second cells; and

15 (d) comparing the polynucleotides identified in step (c) with the polynucleotides motifs identified in step (b) to identify the fragment of the gene encoding the antigen recognized by the immune effector cell.

20 2. The method of identifying a fragment of one or more genes encoding an antigen recognized by an immune effector cell, comprising:

(a) generating a first set of polynucleotide primers corresponding to peptide sequence motifs displayed by an antigen expressing cell and recognized by immune effector cells in a manner restricted by the major histocompatibility complex of the antigen expressing cell;

25 (b) generating a second set of polynucleotide primers corresponding to the unique identifier sequences (SAGE tags) of transcripts differentially expressed in the same antigen expressing cells as described in part (a) above when compared to other antigen expressing cells that are not recognized by the same immune

DIGITAL IMAGE FILE NUMBER 0260

effector cells as in part (a) above but possess a compatible major histocompatibility complex;

5 (c) combining the first and second sets of primers in a combinatorial fashion and identifying the transcripts representative of one or more genes encoding antigens recognized by immune effector; and

10 (d) amplifying cDNA obtained from the first group of cells recognized by the immune effector cells with the combined primers and analyzing the amplified products, thereby identifying one or more fragments of genes encoding antigens recognized by immune effector cells.

15 3. The method of claim 1, wherein step (c) comprises:

(a) providing complementary deoxyribonucleic acid (cDNA) polynucleotides from an antigen expressing cell recognized by the immune effector cells;

20 (b) providing cDNA polynucleotides from cells having a compatible major histocompatibility complex (MHC) to the cells of step (a) but which do not express antigen;

(c) determining and analyzing the cDNAs that are aberrantly expressed by the first cells as compared to the second cells.

25 4. The method according to claim 1, wherein step (c) is performed before step (b).

5. The method according to claim 1 or 2, wherein the immune

effector cells are cytotoxic T lymphocytes (CTLs).

25 6. The method according to claim 5, wherein the CTLs are selected from a group consisting of polyclonal T cells isolated from one individual,

SEARCHED - ATTACHED - FILED

polyclonal T cells isolated from two or more individuals sharing the same MHC restriction, two or more CTLs or any combination thereof.

5           7.       The method according to claim 5, wherein the CTLs recognize an antigen expressed on a neoplastic cell.

10          8.       The method according to claim 7, wherein the neoplastic cell is a tumor cell.

15          9.       The method according to claims 1 or 2, wherein the antigen recognized by the immune effector cell is expressed on a cell site selected from the group consisting of a site of viral infection, a site of autoimmune infiltration, a site of transplantation rejection, a site of inflammation or a site of lymphocyte or leukocyte infiltration.

20          10.      The method according to claim 1, wherein the antigen presenting cell is selected from the group consisting of a cells having a purified MHC class I molecule complexed to a  $\beta_2$ -microglobulin; an intact antigen presenting cell; and a foster antigen presenting cell.

25          11.      The method according to claim 1, wherein the first cell that expresses the antigen recognized by an immune effector cell is a foster antigen presenting cell.

25          12.      The method according to claim 1, wherein the second cell that does not express antigen is a foster antigen presenting cell that lacks antigen processing activity and expresses MHC molecules free of bound peptides.

000000000000000000000000

00000000-0000-0000-0000-000000000000

13. The method according to claims 1 or 2, further comprising isolating the gene encoding the antigen recognized by immune effector cells.

5        14. A method of administering a vaccine comprising administering to a subject an effective amount of a first protein comprising a polypeptide encoded by the gene prepared by the method of claim 13.

10      15. A method of claims 1 or 2, further comprising inserting the gene into a suitable host cell and administering an effective amount of the transduced host cell to a subject.

15      16. A method of claims 1 or 2, further comprising expression cloning of the gene encoding the antigen.

20      17. The method of claim 13, further comprising administering an effective amount of the gene to a subject.

18. The method of claim 14, further comprising coadministering an effective amount of a cytokine.

25      19. The method of claim 14, further comprising coadministering an effective amount of a co-stimulatory molecule.

20. A polynucleotide fragment identified by the method of claim 1.

21. A host cell comprising the polynucleotide fragment of claim 20.

22. A vector comprising the polynucleotide fragment of claim 20.

23. A gene comprising the polynucleotide fragment of claim 1.

24. A polynucleotide encoded by the polynucleotide fragment of claim  
20.